

# Hepatoprotective effects of systemic ER activation

Plotting lipid quantification and Seahorse data

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```
library(tidyverse)
source("code/00_helper_functions.R")
```

## AML12 lipid droplet quantification, Figure 5C

```
aml12_t1 <- read.delim("data/LD_quant_aml12_trial1.txt")
aml12_t1_grp <- aml12_t1 %>% group_by(condition) %>% dplyr::select(2:7) %>% mutate(Trial = "Trial1")

aml12_t2 <- read.delim("data/LD_quant_aml12_trial2.txt")
aml12_t2_grp <- aml12_t2 %>% group_by(condition) %>% dplyr::select(2:7) %>% mutate(Trial = "Trial2")

combined_t1_t2 <- rbind(aml12_t2_grp, aml12_t1_grp)

# Determine median of siNT for all four parameters
combined_t1_t2_medianNorm <- combined_t1_t2 %>% ungroup() %>% group_by(Trial, condition) %>%
  mutate(LD_per_cell_median = median(LD_per_cell)) %>%
  dplyr::select(condition, Trial, LD_per_cell_median) %>%
  filter(condition=="siNT") %>%
  unique()

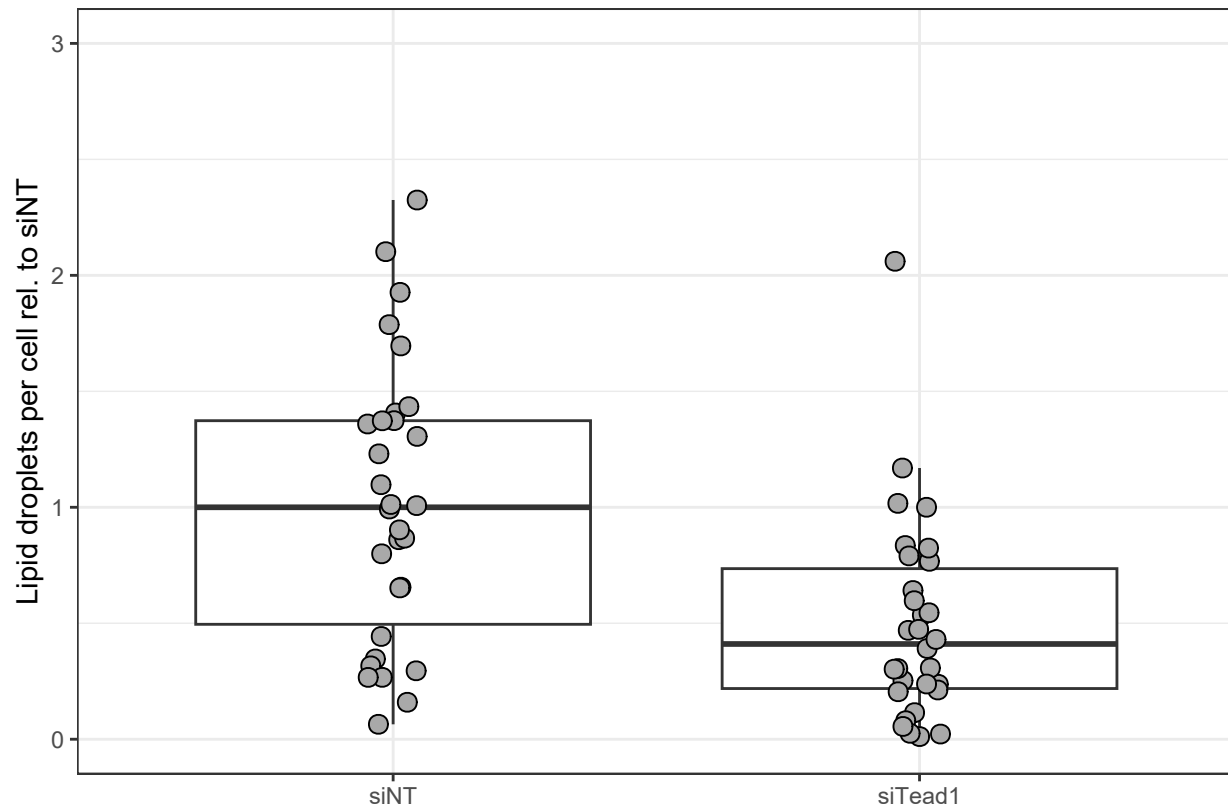
# Split per trial
combined_t1_t2_medianNorm_trial1 <- combined_t1_t2_medianNorm %>% filter(Trial=="Trial1")
combined_t1_t2_medianNorm_trial2 <- combined_t1_t2_medianNorm %>% filter(Trial=="Trial2")

# Normalize the values, per trial
aml12_t1_grp.norm <- aml12_t1_grp %>%
  mutate(LD_per_cell_norm = LD_per_cell/combined_t1_t2_medianNorm_trial1$LD_per_cell_median)

aml12_t2_grp.norm <- aml12_t2_grp %>%
  mutate(LD_per_cell_norm = LD_per_cell/combined_t1_t2_medianNorm_trial2$LD_per_cell_median)

combined_t1_t2_norm <- rbind(aml12_t2_grp.norm, aml12_t1_grp.norm)

ggplot(combined_t1_t2_norm, aes(condition, LD_per_cell_norm)) +
  coord_cartesian() +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(shape=21, size=3, width=0.05, fill="darkgrey") +
  #geom_jitter(shape=21, size=3, width=0.05, aes(fill=factor(Trial))) +
  theme_bw() +
  scale_y_continuous(limits=c(0,3)) +
  ylab("Lipid droplets per cell rel. to siNT") +
  xlab("")
```



```
# Perform normality tests on the data distribution. First, separate the two conditions
combined_t1_t2_norm_siNT <- combined_t1_t2_norm %>% filter(condition=="siNT")
combined_t1_t2_norm_siTead1 <- combined_t1_t2_norm %>% filter(condition=="siTead1")

shapiro.test(combined_t1_t2_norm_siNT$LD_per_cell_norm) # 0.4 --> normal
```

```
##
## Shapiro-Wilk normality test
##
## data: combined_t1_t2_norm_siNT$LD_per_cell_norm
## W = 0.96488, p-value = 0.4101
shapiro.test(combined_t1_t2_norm_siTead1$LD_per_cell_norm) # 0.001 --> not normal
```

```
##
## Shapiro-Wilk normality test
##
## data: combined_t1_t2_norm_siTead1$LD_per_cell_norm
## W = 0.86004, p-value = 0.001013
# perform both wilcoxon and t-test, since one condition is normally distributed and one is not.
```

```
wilcox.test(combined_t1_t2_norm$LD_per_cell_norm ~ combined_t1_t2_norm$condition) # p = 0.000258
```

```
##
## Wilcoxon rank sum exact test
##
## data: combined_t1_t2_norm$LD_per_cell_norm by combined_t1_t2_norm$condition
## W = 691, p-value = 0.000258
## alternative hypothesis: true location shift is not equal to 0
t.test(combined_t1_t2_norm$LD_per_cell_norm ~ combined_t1_t2_norm$condition) # p = 0.000399
```

```
##
## Welch Two Sample t-test
##
## data: combined_t1_t2_norm$LD_per_cell_norm by combined_t1_t2_norm$condition
## t = 3.7811, df = 52.856, p-value = 0.000399
## alternative hypothesis: true difference in means between group siNT and group siTead1 is not equal to 0
## 95 percent confidence interval:
## 0.2411529 0.7861210
```

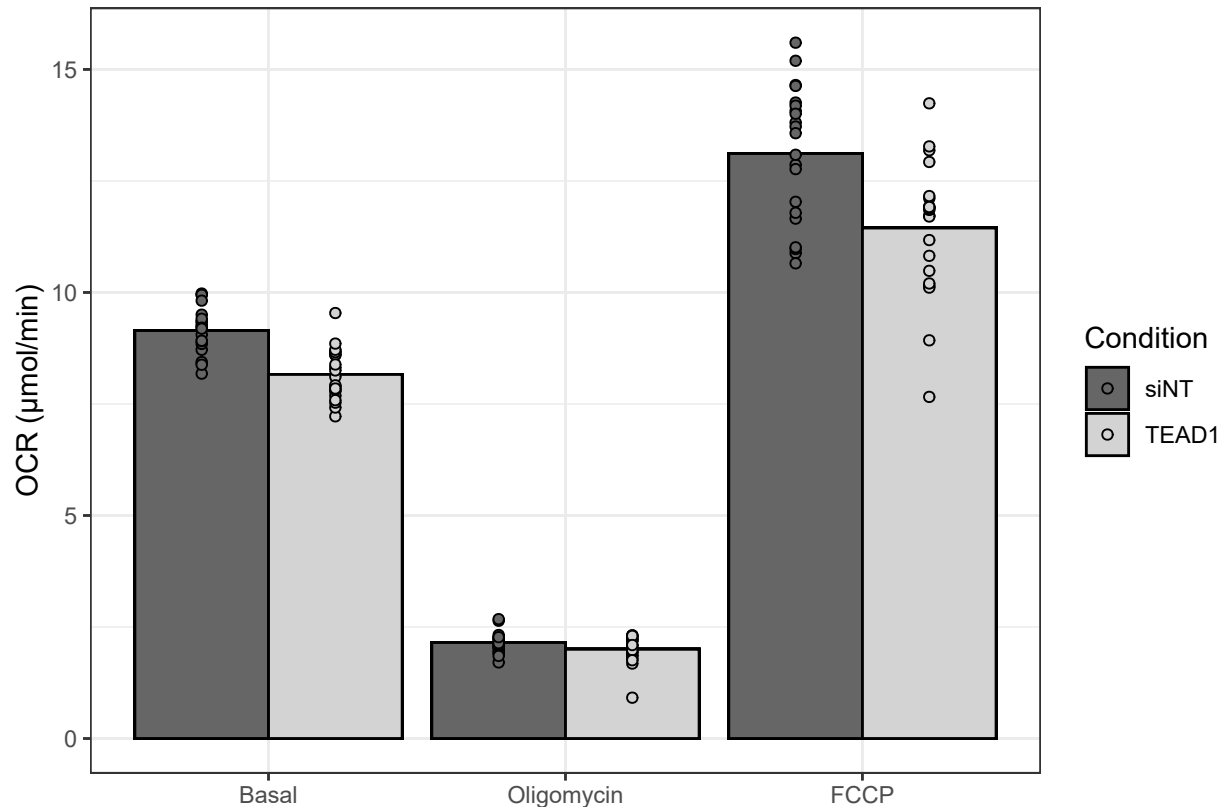
```
## sample estimates:
##      mean in group siNT mean in group siTead1
##      1.0107544          0.4971175
```

## Seahorse, Figure 5D

```
data <- read.delim("data/siTEAD1_Seahorse")

library(dplyr)
library(ggplot2)
data.grouped <- data %>% mutate(OCR_umol_per_l = Value/1000) %>% group_by(Condition, SeahorseStage) %>% mutate(mean=mean(OCR_umol_per_l))

order = c("Basal", "Oligomycin", "FCCP")
ggplot(data.grouped, aes(x=factor(SeahorseStage, levels=order), y=mean, fill=Condition)) +
  geom_col(position=position_dodge(width=0.9), color="black") +
  geom_point(position=position_dodge(width=0.9), shape=21, color="black", aes(y=OCR_umol_per_l)) +
  theme(axis.text=element_text(size=18)) +
  theme_bw() +
  ylab("OCR (μmol/min)") +
  xlab("") +
  scale_fill_manual(values=c("#666666", "lightgrey"))
```



```
data.grouped.basal <- data.grouped %>% filter(SeahorseStage == "Basal")
data.grouped.oligo <- data.grouped %>% filter(SeahorseStage == "Oligomycin")
data.grouped.FCCP <- data.grouped %>% filter(SeahorseStage == "FCCP")
t.test(data.grouped.basal$Value ~ data.grouped.basal$Condition)
```

```
##
## Welch Two Sample t-test
##
## data: data.grouped.basal$Value by data.grouped.basal$Condition
## t = 5.7637, df = 39.799, p-value = 1.032e-06
## alternative hypothesis: true difference in means between group siNT and group TEAD1 is not equal to 0
## 95 percent confidence interval:
## 638.6348 1328.5404
## sample estimates:
```

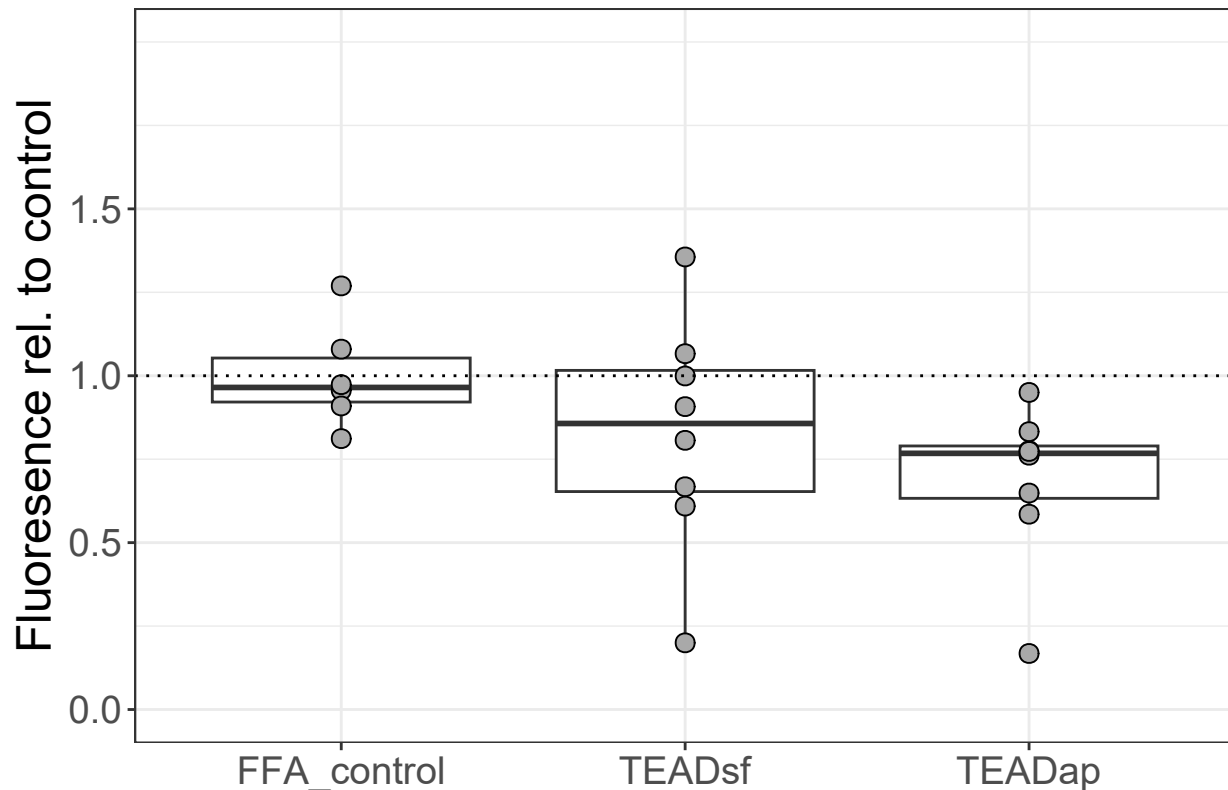
```
## mean in group siNT mean in group TEAD1
##          9146.080          8162.493
t.test( data.grouped.oligo$Value ~ data.grouped.oligo$Condition)

##
## Welch Two Sample t-test
##
## data: data.grouped.oligo$Value by data.grouped.oligo$Condition
## t = 1.7832, df = 36.325, p-value = 0.08291
## alternative hypothesis: true difference in means between group siNT and group TEAD1 is not equal to 0
## 95 percent confidence interval:
## -20.77652 324.15494
## sample estimates:
## mean in group siNT mean in group TEAD1
##          2151.737          2000.048
t.test( data.grouped.FCCP$Value ~ data.grouped.FCCP$Condition)

##
## Welch Two Sample t-test
##
## data: data.grouped.FCCP$Value by data.grouped.FCCP$Condition
## t = 3.5835, df = 40, p-value = 0.0009102
## alternative hypothesis: true difference in means between group siNT and group TEAD1 is not equal to 0
## 95 percent confidence interval:
## 728.286 2612.386
## sample estimates:
## mean in group siNT mean in group TEAD1
##          13114.86          11444.53
```

## PHH spheroid lipid quantification, Figure 5E

```
inhibitors_adipored <- read.csv("data/TEAD_inhibitors_adipored.csv")
order <- c("FFA_control", "TEADsf", "TEADap")
ggplot(inhibitors_adipored, aes(x=factor(inhibitor, levels=order), y=norm_to_ctrl)) +
  geom_boxplot() +
  geom_point(aes(x=inhibitor, y=norm_to_ctrl), size=3, fill="darkgrey", shape=21) +
  theme_bw() +
  theme(text = element_text(size=18)) +
  ylab("Fluorescence rel. to control") +
  xlab("") +
  scale_y_continuous(limits=c(0,2), breaks=c(0,0.5,1, 1.5)) +
  geom_hline(yintercept = 1, linetype="dotted")
```



```
# Statistics
inhibitors_TEADap <- inhibitors_adipored %>% filter(!inhibitor=="TEADsf")
inhibitors_TEADsf <- inhibitors_adipored %>% filter(!inhibitor=="TEADap")

t.test(inhibitors_TEADap$value ~ inhibitors_TEADap$inhibitor)

##
## Welch Two Sample t-test
##
## data: inhibitors_TEADap$value by inhibitors_TEADap$inhibitor
## t = 2.962, df = 11.907, p-value = 0.01197
## alternative hypothesis: true difference in means between group FFA_control and group TEADap is not equal to 0
## 95 percent confidence interval:
##  0.400637 2.637117
## sample estimates:
## mean in group FFA_control      mean in group TEADap
##      4.850106              3.331229

t.test(inhibitors_TEADsf$value ~ inhibitors_TEADsf$inhibitor)

##
## Welch Two Sample t-test
##
## data: inhibitors_TEADsf$value by inhibitors_TEADsf$inhibitor
## t = 1.2531, df = 10.318, p-value = 0.2378
## alternative hypothesis: true difference in means between group FFA_control and group TEADsf is not equal to 0
## 95 percent confidence interval:
## -0.6484695  2.3312236
## sample estimates:
## mean in group FFA_control      mean in group TEADsf
##      4.850106              4.008729
```

## SessionInfo

```
sessionInfo()

## R version 4.2.1 (2022-06-23 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
```

```

## Running under: Windows 10 x64 (build 19045)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_United States.utf8
## [2] LC_CTYPE=English_United States.utf8
## [3] LC_MONETARY=English_United States.utf8
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.utf8
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods   base
##
## other attached packages:
## [1] lubridate_1.9.2 forcats_1.0.0  stringr_1.5.0  dplyr_1.1.0
## [5] purrr_1.0.1    readr_2.1.4    tidyr_1.3.0    tibble_3.2.1
## [9] ggplot2_3.4.2  tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
## [1] highr_0.10      pillar_1.9.0    compiler_4.2.1  tools_4.2.1
## [5] digest_0.6.30   timechange_0.2.0 evaluate_0.21    lifecycle_1.0.3
## [9] gtable_0.3.3    pkgconfig_2.0.3 rlang_1.1.1     cli_3.4.1
## [13] rstudioapi_0.15.0 yaml_2.3.7      xfun_0.39       fastmap_1.1.0
## [17] withr_2.5.0     knitr_1.43      generics_0.1.3  vctrs_0.6.2
## [21] hms_1.1.3       grid_4.2.1      tidyrselect_1.2.0 glue_1.6.2
## [25] R6_2.5.1        fansi_1.0.3     rmarkdown_2.23  farver_2.1.1
## [29] tzdb_0.4.0      magrittr_2.0.3  scales_1.2.1    htmltools_0.5.5
## [33] colorspace_2.0-3 labeling_0.4.2  utf8_1.2.2      stringi_1.7.8
## [37] munsell_0.5.0

```